

Novel nanobodies discriminate CD33 full-length and CD33ΔE2 isoforms: towards improved CD33 phenotyping and targeting in acute myeloid leukemia

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INTRODUCTION

CD33 is a transmembrane protein expressed on **myeloid cells** and is described as an inhibitory receptor of the cell activation. It became a **target of interest in acute myeloid leukemia** (AML) as it is expressed on a large majority of blasts. The full-length (**CD33-FL**) protein is composed of two extracellular domains ; the IgV and the IgC domains. Another truncated isoform of the protein (**CD33-E2**), is **lacking the IgV domain**. However, most antibodies recognizes an epitope located on the IgV domain, including the antibody-drug conjugate Gemtuzumab Ozogamicin, raising hypothesis of resistance linked to the CD33-E2 isoform expression. As mentioned in a previous study, antibody clone HIM3-4 can recognize both CD33-FL and CD33-E2 isoforms¹, however more recent studies are critical on this point, suggesting that HIM3-4 can poorly bind CD33-E2^{2,3,4}. In this work, we combined flow cytometry, BioLayer Interferometry and structural analysis to propose a model describing **two conformational states of the IgC domain**, explaining the recognition profile of HIM3-4. To support this hypothesis, we developed a nanobody, NVD_110, able to detect **only the CD33-E2 isoform**.

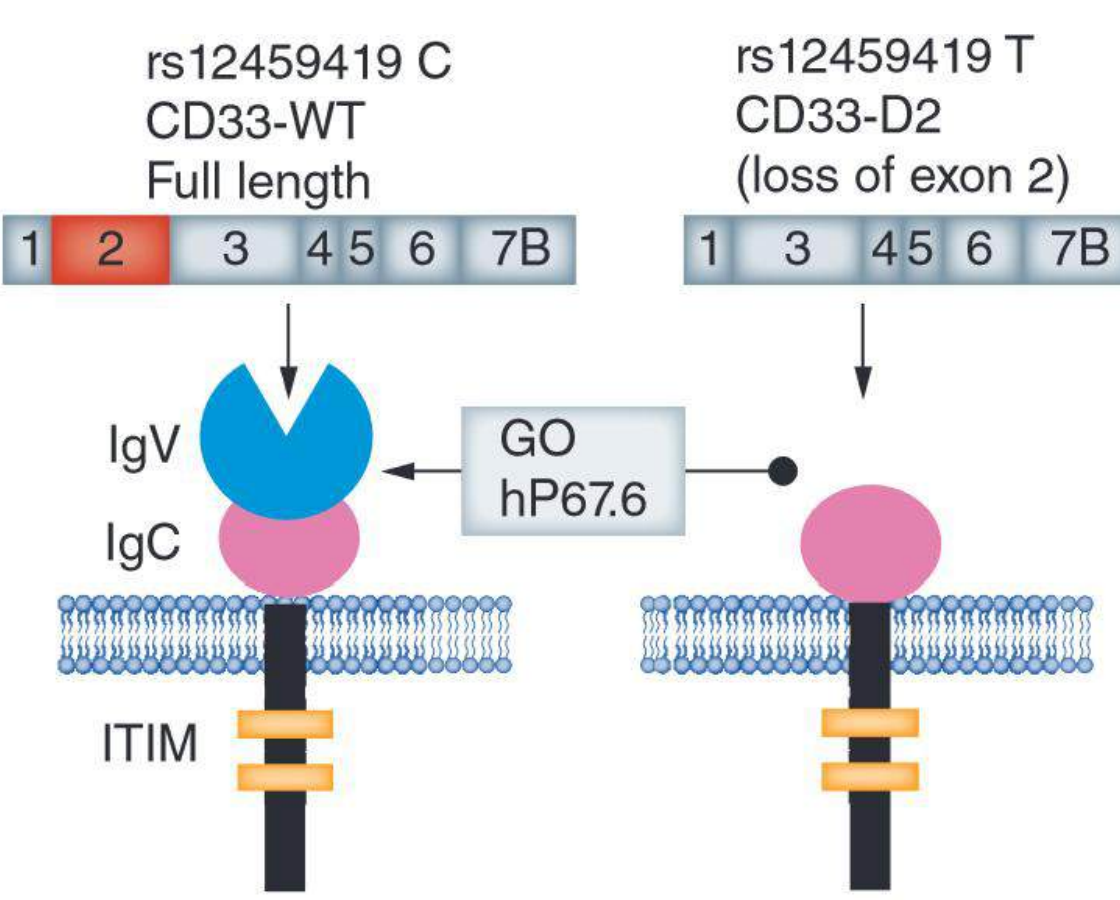


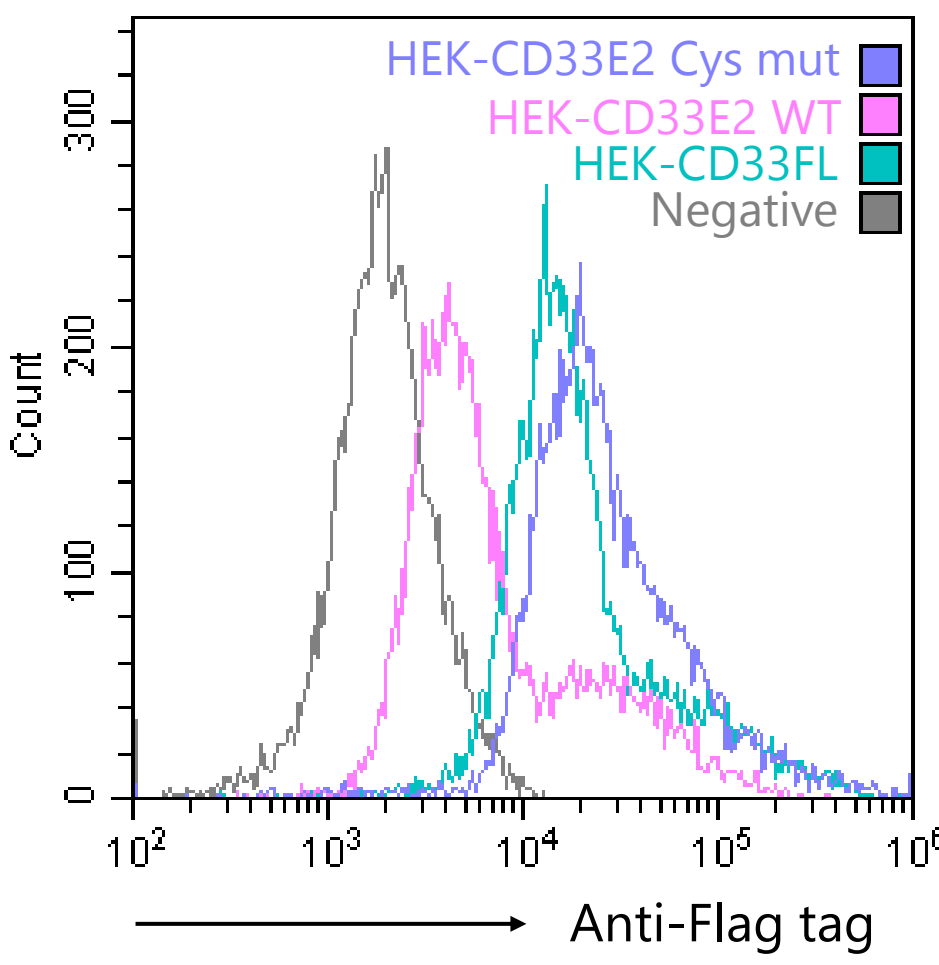
Figure from Gbadamosi et al., *Future Oncol.* (2018)

EXPERIMENTAL STRATEGY

Model : Transfected HEK 293 Ebna cells

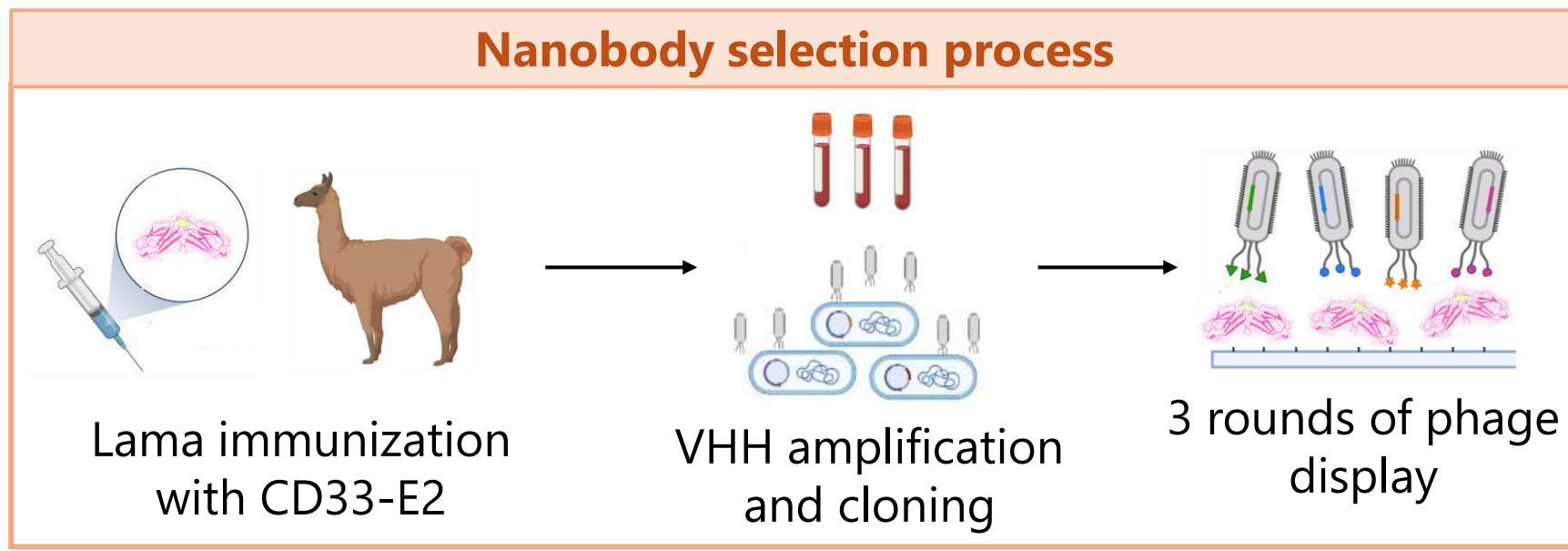
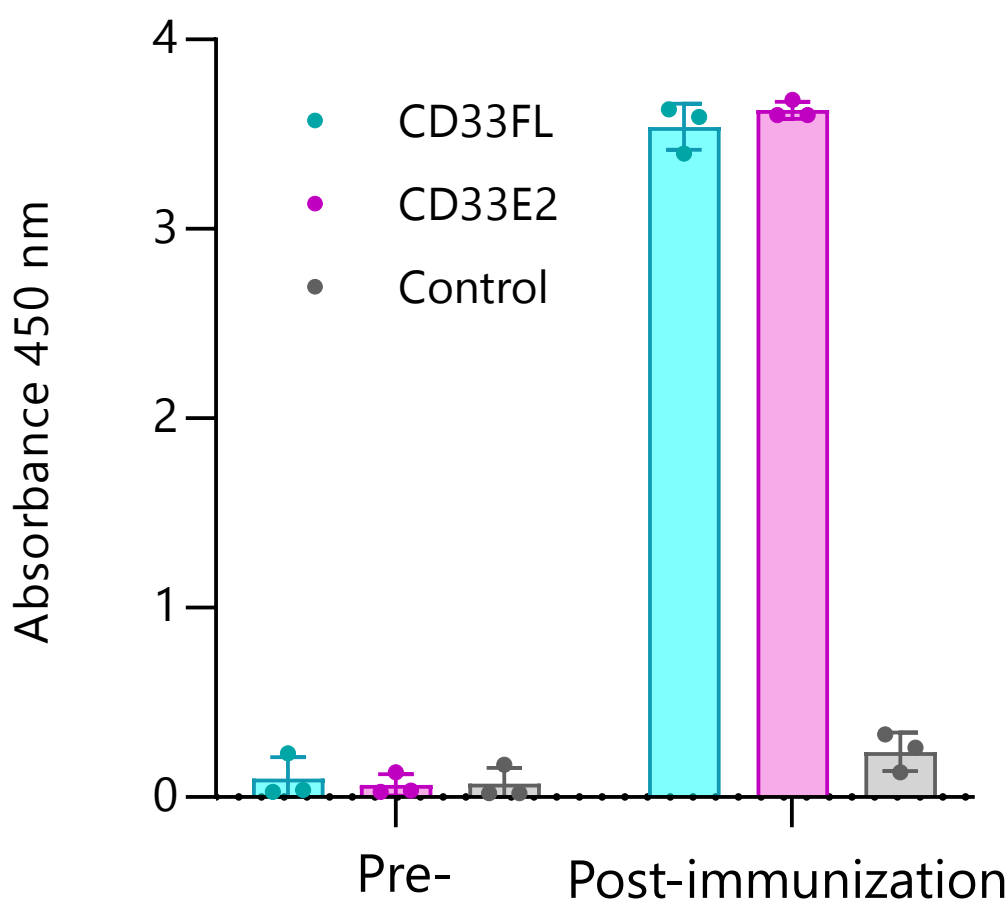
Figure 1. CD33 isoform transfection is confirmed by flow cytometry. Transfection efficacy depends on the isoform.

Flow cytometry signal was normalized using a Flag-tag.



Nanobody selection : Phage display from lama immune bank

Figure 2. ELISA serum on recombinant soluble ectodomains. The serum can respectively recognize both CD33-FL and CD33-E2 isoforms, confirming the immunization success.



RESULTS

The detection of CD33-E2 is limited due to a lack of specific antibody

HIM3-4 clone characterization : specificity and affinity

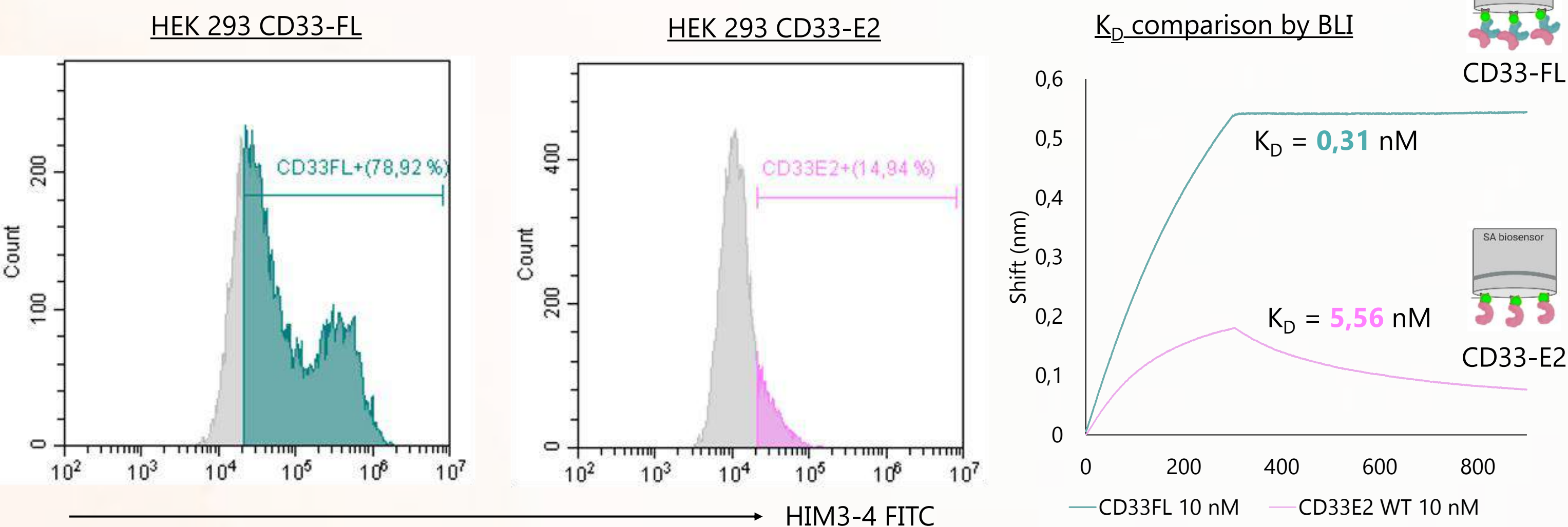


Figure 3. HIM3-4 specificity and affinity for CD33 isoforms measured by flow cytometry on HEK transfected cells and BioLayer Interferometry (BLI) on soluble recombinant ectodomains.

HIM3-4 displays an equivalent kinetic profile on soluble ectodomains of CD33-E2 WT and CD33 E2 Cysteine-mutant, while recognition of the membrane proteins is different, suggesting an **issue of accessibility**.

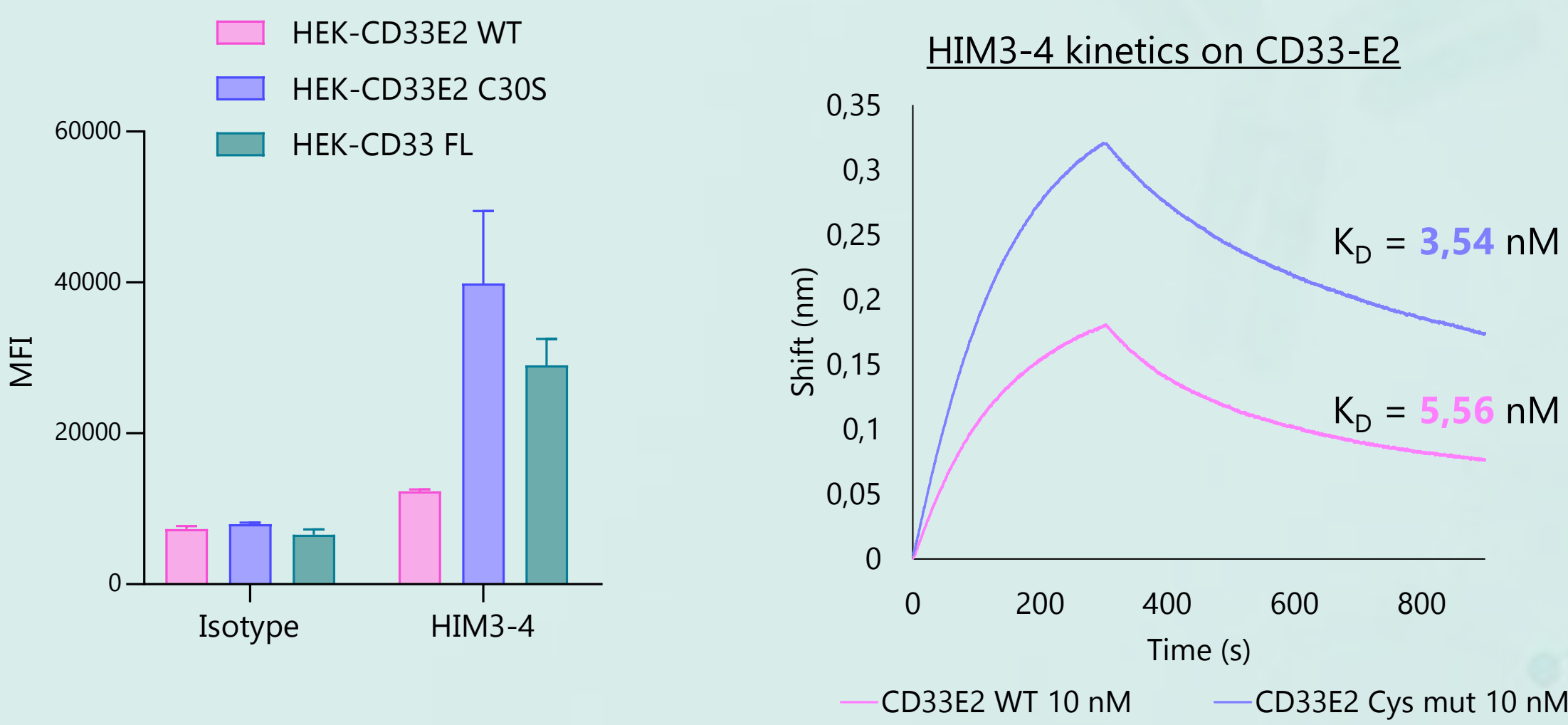


Figure 5. Detection profile of HIM3-4 on CD33 isoforms, including a mutated version of CD33-E2, by flow cytometry and BLI.

NVD_110 : a new CD33-E2-specific nanobody

NVD_110 is a **VHH-Fc mouse fusion**.

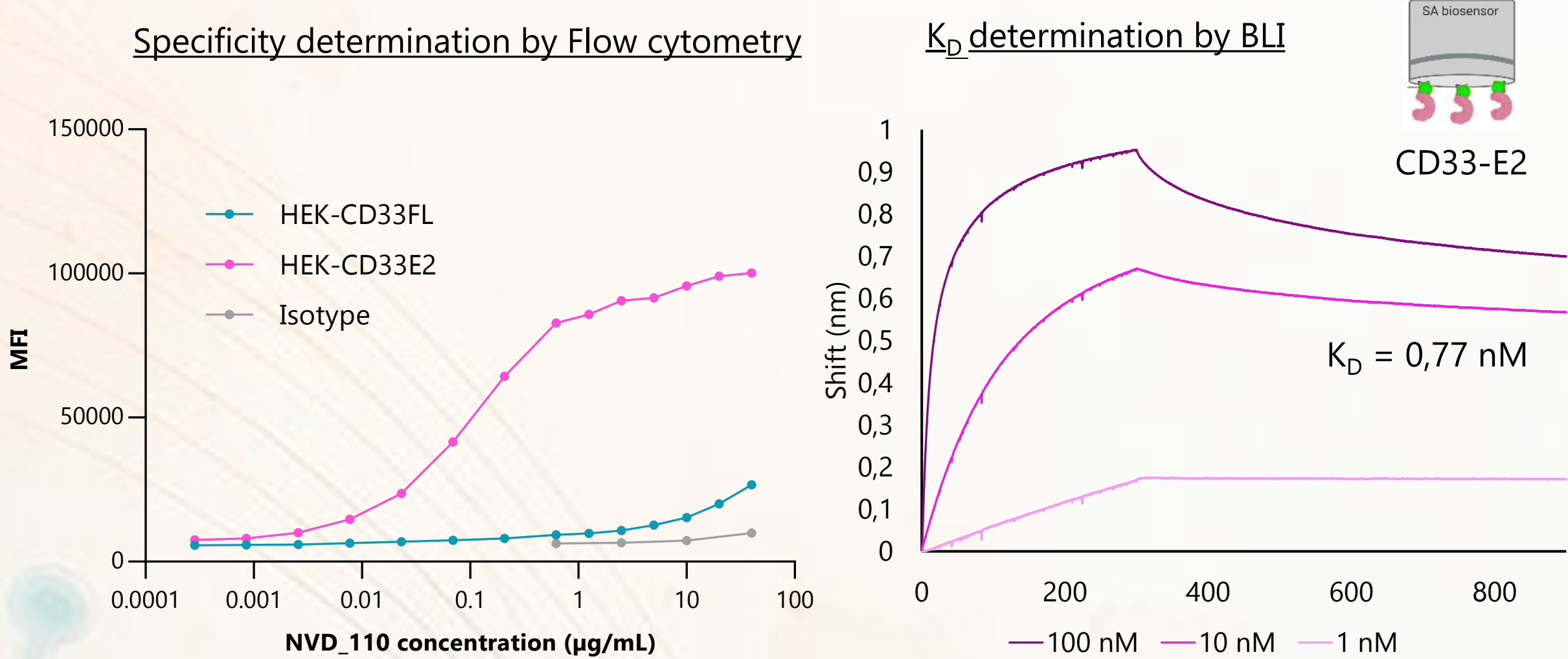


Figure 4. Titration curves of NVD_110-ATTO488 on transfected cells by flow cytometry and determination of kinetic parameters on the recombinant soluble ectodomain of CD33-E2 by BLI.

Conformational switch hypothesis and antibody affinity : how isoform structural dynamics can influence epitope accessibility and recognition

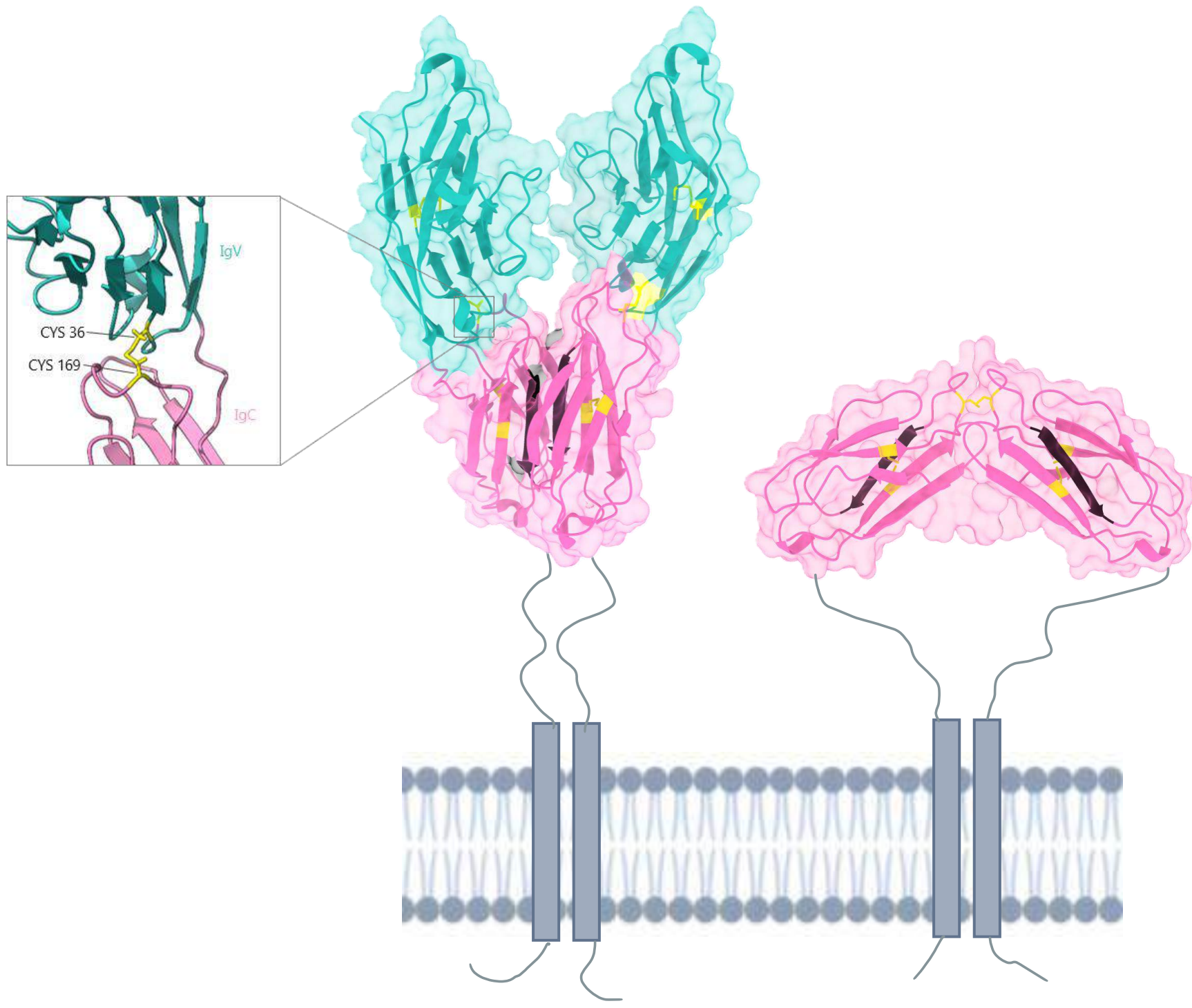


Figure 6. Conformational switch hypothesis. On the CD33-E2 isoform, the Cys169 forming a disulfide bond with Cys36 in the CD33-FL isoform is free, leading to a homodimerization via a disulfide bond. This conformational switch creates two states of the IgC : the CD33-FL state and the CD33-E2 state, possibly creating or masking epitopes in one or the other state, for example for the region coloured in black.

CONCLUSION

HIM3-4, targeting IgC domain, is not a relevant antibody to detect the truncated isoform CD33-E2. The nanobody **NVD_110** has an interesting capacity to detect only CD33-E2 at low concentration, offering new opportunities for the detection of CD33-E2 in patients with AML. These data, combined with protein engineering on the CD33-E2 isoform, lead to the hypothesis of a conformational switch of the IgC domain, raising the importance to consider structural analysis when choosing an antibody for flow cytometry experiments.

REFERENCES

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- ²Godwin et al., The CD33 splice isoform lacking exon 2 as therapeutic target in human acute myeloid leukemia, *Leukemia* (2020)
- ³Gbadamosi et al., Novel CD33 antibodies unravel localization, biology and therapeutic implications of CD33 isoforms, *Future Oncol.* (2021)
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